

Molecular Characterization of Potential Microcystin-Producing Cyanobacteria in Lake Ontario Embayments and Nearshore Waters[▽]

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The distribution and genotypic variation of potential microcystin (MC) producers along the southern and eastern shores of Lake Ontario in 2001 and 2003 were examined using a suite of PCR primers. Cyanobacterial, *Microcystis* sp., and *Microcystis*-specific toxin primer sets identified shoreline distribution of cyanobacterial DNA (in 97% of the stations) and MC synthetase genes (in 50% of the stations). Sequence analysis of a partial *mcyA* amplicon targeting *Microcystis*, *Anabaena*, and *Planktothrix* species indicated that the *Microcystis* sp. genotype was the dominant MC genotype present and revealed a novel *Microcystis*-like sequence containing a 6-bp insert. Analysis of the same samples with genus-specific *mcyE* primers confirmed that the *Microcystis* sp. genotype was the dominant potential MC producer. Genotype compositions within embayments were relatively homogenous compared to those for shoreline and tributary samples. MC concentrations along the shoreline exhibited both temporal and spatial differences as evidenced by the protein phosphatase inhibition assay, at times exceeding the World Health Organization guideline value for drinking water of 1.0 µg MC-LR_{eq} liter⁻¹. MC genotypes are widespread along the New York State shoreline of Lake Ontario, appear to originate nearshore, and can be carried through the lake via wind and surface water current patterns.

Lake Ontario is the easternmost and smallest of the Great Lakes, bordering Ontario, Canada, and New York, United States. Over 8 million people live in the watershed area and rely on Lake Ontario for recreational and drinking water. Approximately 80% of the water is supplied by the Niagara River, flowing in from Lake Erie, while 14% is delivered by small tributaries. The major outflow (93%) is via the St. Lawrence River (11). Lake Ontario, located last in the chain of Great Lakes, is affected by human activities occurring throughout the water basins of Lakes Superior, Michigan, Huron, and Erie. The Lake Ontario drainage basin is one of the fastest-growing areas in North America in terms of population and one of the most sprawling regions in the world. This growth is concurrent with the alteration of shoreline dynamics, resulting in increased nutrient and contaminant runoff, the effects of which are amplified by the large catchment area (64,030 km²) and long residence time (6 years) (10). Surface water currents controlled by the prevailing winds transport nutrients and particles along the Lake Ontario shoreline in a counterclockwise manner, while a central clockwise gyre mixes particles from the northern to the southern shores (Fig. 1) (3). The combination of runoff and mixing can result in the eutrophication of Lake Ontario embayments needed to trigger massive algal blooms, often occurring in late summer months with concomitant toxin production by cyanobacteria.

Cyanobacteria produce a number of potent toxins. The most common are the microcystins (MCs), hepatotoxins made by a wide range of cyanobacteria, including *Microcystis*, *Anabaena*,

Nostoc, and *Planktothrix* species. The generic structure of an MC is cyclo(–D-Ala–X–D-MeAsp–Z–Adda–D-Glu–Mdha–), where Mdha is *N*-methyldehydro-alanine, D-MeAsp is D-erythro-β-methylaspartic acid, and Adda is the nonproteogenic amino acid 3-amino-9-methoxy-10-phenyl-2,6,8-trimethyl-decca-4,6-dienoic acid (31). Structural variation can occur at all seven positions; however, the X and Z positions represent highly variable amino acids, and demethylation of MeAsp and Mdha is common (42). There are currently more than 80 identified MC variants, each possessing different degrees of toxicity, ranging from 50 to >1,200 µg kg⁻¹ body weight (the 50% lethal dose [intraperitoneal] for mouse) (2, 42, 53).

Toxic and nontoxic populations of cyanobacteria can coexist in a single ecosystem and are indistinguishable by microscopy, rendering this technique unreliable for determining potential MC production. Methods commonly used for toxin monitoring include high-performance liquid chromatography (LC) with photodiode array (PDA) detection and/or mass spectrometry (MS), a protein phosphatase inhibition assay (PPIA), and enzyme-linked immunosorbent assays. These techniques are based on the structures or activities of MCs.

Elucidation of the MC synthetase gene cluster (*mcy*) encoding the nonribosomal peptide synthetase polyketide synthase enzyme complex has allowed the development of molecular techniques for studying MC-producing genera. The *mcy* gene clusters are significantly different between the major MC-producing genera *Microcystis*, *Anabaena*, and *Planktothrix* (8, 38, 45). Differences are found in the organization and presence of the *mcy* genes as well as the nucleotide sequences. The result of this genetic diversity is not fully understood, but its origin may be attributed to the ancestral nature of the genes, transposition between genera, and inter- and intrastrain genetic

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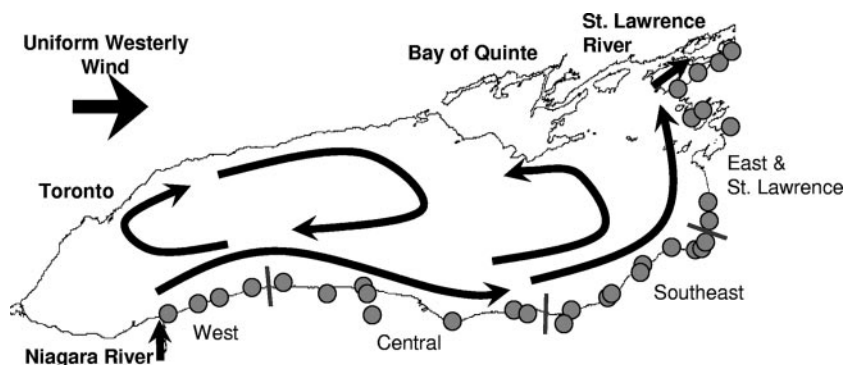


FIG. 1. Sampling locations along the New York State border of Lake Ontario separated into regions. Arrows represent the Lake Ontario average annual surface water current pattern. (Adapted from reference 3 with permission of the publisher.)

recombination (27, 28, 35, 44, 45). Multiple studies have focused on detection of the *mcy* genes using a PCR approach with species-specific primers based on differences within the *mcy* gene clusters (18, 20, 26, 32, 36, 47) as well as universal primers targeting conserved sequences of the MC operon (14, 17). The common *mcy* genes targeted are *mcyA*, *-B*, *-C*, *-D*, and *-E*, with many studies using a combination of the genes (15, 26, 27, 33, 44, 48). Several studies have indicated that MC-producing *Microcystis* spp. exhibit substantial genetic diversity within and between lakes located in both similar and different geographic locations (37, 52, 54). Examination of the *mcy* genes can give information on MC potential, the diversity of toxin producers within a population, and the geographic distribution of MC-producing genotypes.

Here, we investigated the occurrence and diversity of MC genotypes from embayments and lakeside water samples along the southern and eastern shores of Lake Ontario in 2001 and 2003. Previous studies have addressed the occurrence of MCs in Lake Ontario using analytical and biochemical techniques (5, 24); however, this is the first report on the identification of MC producers along the New York shoreline of Lake Ontario using molecular techniques. Multiple MC-specific PCR primers and DNA sequencing were used to determine the potential for MC production and toxin genotype composition in embayments and shoreline samples open to the lake. This was compared to MC concentration determined by PPIA. MC variant, trophic status, and cyanobacterial species composition were explored to determine the relationship with genotype composition and MC production. These data were used to identify areas of potential MC production and to address the contribution of embayments and surface water current patterns to the distribution of MCs along the New York shoreline of Lake Ontario.

MATERIALS AND METHODS

Sample collection. Samples were collected during the summers of 2001 and 2003 from the southern and eastern shores of Lake Ontario (32 stations in 2001, 8 stations in 2003 [Fig. 1]). At each station, water samples were collected from a depth of 1.0 m, filtered, and immediately stored on dry ice for later analysis in the laboratory. Toxin samples (up to 20 liters) were filtered through a 90-mm glass fiber filter (Whatman 934-AH). DNA and chlorophyll samples (up to 1.0 liter) were filtered onto 47-mm glass fiber filters (Whatman 934-AH). Whole water samples were collected for total phosphorus analysis in acid-washed 125-ml bottles and stored on ice. Surface samples of cyanobacteria were collected with

a 63- μ m plankton net and stored in 0.2% (vol/vol) glutaraldehyde for phylogenetic analysis.

MC analysis. Toxin filters were extracted by sonication in 10 ml of 50% aqueous methanol acidified to 1% (vol/vol) with acetic acid. Extracts were clarified by centrifugation at $27,000 \times g$ followed by filtration through a 0.45- μ m nylon syringe filter and stored at -20°C . MC concentrations were determined using a PPIA, modified from Carmichael and An (7), run in 96-well plates (15). MC variants were identified from concentrated samples (10 to $20\times$) by high-performance LC (Ace 5 C_{18} column, 4.6 by 250 mm) with PDA and MS detectors using a gradient of 30 to 70% acetonitrile in water, both acidified to 0.1% (vol/vol) with trifluoroacetic acid. PDA detection was at 239 nm, and MS with electrospray ionization was used to detect molecular ions between 800 and 1,200 AMU (13). Putative MC variants were compared to standards, published molecular weights, and UV spectra (21, 55).

Chlorophyll *a*, total phosphorous, and phylogenetic analysis. Chlorophyll *a* was determined after extraction by sonication in 15 ml of 90% acetone, using UV-visible light spectrometry in 2001 (34) and fluorescence in 2003 (50). Total phosphorous was determined by acid hydrolysis and ammonium peroxydisulfate digestion, followed by color development with ammonium molybdate, potassium tartrate, and ascorbic acid and detection at 650 nm (9). The trophic statuses of the embayments were determined based on the average of the chlorophyll *a* and total phosphorous trophic status index values calculated using the Carlson trophic status formulas (6).

Cell counts were estimated for samples where we cloned the *mcyA* amplicon. Cyanobacteria were identified to the genus level based on Whitford and Schumacher (51) using a phase contrast light microscope under $\times 100$ to 500 magnification. Cyanobacterial colonies and filaments were counted using a Palmer-Malony counting chamber. Cell numbers were estimated from colony and filament size classes using the protocol of Watzin et al. (49).

DNA analysis. DNA was extracted from a 1.1-cm-diameter subsample of the original 47-mm filter using a protocol modified from Rudi et al. (39) described previously (15). Briefly, filters were placed in Tris-EDTA buffer (pH 8.0) and digested with lysozyme and RNase A, followed by a proteinase K digestion. Samples were clarified by two phenol-chloroform-isoamyl alcohol (25:24:1) extractions and a final chloroform-isoamyl alcohol (24:1) purification. DNA was precipitated in 95% ethanol overnight (-20°C), washed with 80% ethanol, and redissolved in 75 μ l of Tris-EDTA buffer. Quantitation was done by UV spectroscopy at 260 nm.

Extracted DNA was amplified by PCR, separated by 1.5% (wt/vol) agarose gel electrophoresis, and visualized using ethidium bromide. Primer sequences used for PCR were based on cyanobacterial 16S rRNA (CYA), a *Microcystis* sp. 16S rRNA (MIC), and four toxin biosynthetic genes (*mcyA*, *mcyB*, *mcyD*, and *mcyE*) (Table 1). Samples that were negative by both CYA and MIC primer sets were spiked with lambda DNA and amplified with lambda-specific primers as a positive control for amplification. The *mcyB* and *mcyD* primer sets were used for an initial screening of all the samples. The *mcyA* primer set was used to analyze samples collected in August of 2001 and 2003, and stations positive by *mcyA* were amplified using genus-specific *mcyE* primers. Each 25- μ l PCR contained 1.5 mM MgCl_2 , 200 μM of each deoxynucleoside triphosphate, 0.04 U μl^{-1} *Taq* polymerase (Applied Biosystems), 300 ng μl^{-1} bovine serum albumin, 5 ng μl^{-1} DNA, and 400 nM of each primer. Amplification was performed in an MJ Research PTC-100 thermocycler using the following protocol: initial denatur-

TABLE 1. PCR primer sequences

Target	Primer	Direction ^a	Primer sequence (5'→3')	Reference
Cyanobacterial 16S rRNA	CYA	F R	ACGGGTGAGTAACRCGTRA CTTCAYGYAGGCGAGTTGCAGC	46
<i>Microcystis</i> 16S rRNA	MIC	F R	ATGTGCCGCGAGGTGAAACCTAAT TTACAAAYCCAARRRCCTTCCTCCC	30
<i>Microcystis mcyD</i>	mcyD	F R	GGTTCGCCTGGTCAAAGTAA CCTCGCTAAAGAAGGGTTGA	18
<i>Microcystis mcyB</i>	mcyB	F R	TGGGAAGATGTTCTTCAGGTATCCAA AGAGTGGAACAATATGATAAGCTAC	32
<i>Microcystis</i> , <i>Anabaena</i> , and <i>Planktothrix</i> sp. <i>mcyA</i>	mcyA	F R	AAAAGTGTTTTATTAGCGGCTCAT AAAATTAAGCCGTATCAAA	14
<i>Microcystis</i> , <i>Anabaena</i> , and <i>Planktothrix</i> sp. <i>mcyE</i>	mcyE	F	GAAATTTGTGTAGAAGGTGC	47
<i>Microcystis</i> sp. <i>mcyE</i>		R	CAATGGGAGCATAACGAG	36
<i>Anabaena</i> sp. <i>mcyE</i>		R	CAATCTCGGTATAGCGGC	
<i>Planktothrix</i> sp. <i>mcyE</i>		R	CTCAATCTGAGGATAACGAT	

^a F, forward; R, reverse.

ation at 94°C for 2 min, followed by 20 cycles of 94°C for 30 s, 65°C for 45 s (decreasing by 0.5°C each cycle), and 72°C for 1 min, followed by 15 additional cycles with a steady annealing temperature of 55°C, and ending with a final extension of 72°C for 8 min.

The *mcyA* amplicons were cloned using a TOPO TA cloning kit from Invitrogen Life Technologies with chemically competent pCR 2.1-TOPO TOP10 One Shot *Escherichia coli* cells. Up to 10 separate colonies were picked and grown overnight in liquid LB plus ampicillin (50 µg ml⁻¹) for plasmid miniprep. Minipreps were completed via the alkaline lysis method, and the final DNA pellet was dissolved in 50 µl of water (41). Transformants were confirmed by digestion with EcoRI. DNA sequencing of the genetic recombinants was performed at the Biotechnology Resource Center at Cornell University (Ithaca, NY) using an Applied Biosystems automated 3730 DNA analyzer with BigDye Terminator chemistry and AmpliTaq-FS DNA polymerase. DNA sequences were modified using BioEdit (version 7.0.5.3) and Mega 3.1, queried against known sequences in the GenBank database using a nucleotide-nucleotide BLAST (blastn), and aligned to known sequence matches using ClustalX (version 1.81) and Mega 3.1 (1, 12, 16, 19). Neighbor-joining analysis of the *mcyA* sequence alignment was done with PAUP* 4.0 and Mega 3.1 using *p*-distance analysis (43). Bootstrap values were obtained for 1,000 replicates.

Nucleotide sequence accession numbers. Sequences were deposited in GenBank (accession numbers EF424278 through EF424374).

RESULTS

Occurrence of MCs along the New York coast of Lake Ontario. Low levels of MCs were detected at each station from the sample period of June to August 2001, ranging from 0.002 to 0.070 µg liter⁻¹, with the largest amount occurring at Port Bay East in early August (Table 2). A temporal variation in MC concentrations was observed, with some stations highest in June and others in August, but the highest lakewide average and total MC concentrations occurred during August (data not shown). Stations resampled in August 2003 had MC concentrations ranging from 0.003 to 1.043 µg liter⁻¹, with the largest amount occurring at Selkirk Shores in late August (Table 2). The Oswego Shore station also had a high MC value (0.990 µg liter⁻¹) in early August. In late August 2003, Nine Mile Bay, Little Salmon River, and Selkirk Shores stations all had higher MC concentrations than those observed in late August 2001

(11 to 69 times higher per station). West Sodus Bay, Little Sodus Bay (bayside), and Chaumont Bay all had similar MC concentrations as observed in 2001.

Presence of *mcyB* and *mcyD* genes. Stations sampled in June, July, and August 2001 were analyzed for MC potential with the *mcyB* and *mcyD* primer sets (Table 2) as well as the CYA- and MIC-specific primer sets. In June 2001, the westernmost station, Fort Niagara, was the only station positive by the *mcyB* or *mcyD* primer set. Six stations spanning the entire sampling region were positive for MC potential in July. In early August 2001, there were no toxin genes detected from any of the sampling stations. In late August, MC potential increased to include nine stations spanning all regions except the western, which was not sampled at this time. Regardless, the greatest MC potential was observed in late August.

In late August 2003, eight stations were sampled, and each was positive for MC potential by both the *mcyB* and the *mcyD* primer sets except Chaumont Bay, which had only an *mcyD* amplicon (Table 2). Only one sample was collected earlier in the year (from Oswego Shore, collected in early August); therefore, changes in toxin potential throughout the season could not be determined for 2003.

MC potential, as determined by *mcyB* and *mcyD*, was detected in 25 of the 107 samples collected in 2001 and 2003. In 9 of those 25 samples, only one of the toxin genes was detected, with 8 of those 9 instances being only the *mcyD* amplicon.

Determination of genotype composition by *mcyA* sequencing. To determine the cyanobacterial genera present that could be responsible for MC production, samples collected in August of 2001 were analyzed using an *mcyA* primer set targeting the *mcyA* gene from *Microcystis*, *Anabaena*, and *Planktothrix* species and the resulting amplicons cloned and sequenced (14). The *mcyA* sequences were confirmed to be from the MC synthetase complex by using the GenBank database BLASTX search. Phylogenetic analysis, utilizing reference *mcyA* se-

TABLE 2. Sampling locations along the Lake Ontario shoreline, with numbers of times sampled, maximum MC levels reached, and *mcyB* and *mcyD* potentials for each month

Regional location, yr, and station name	Location type ^a	No. of times sampled	Maximum MC-LR _{eq} (µg/liter) ^b	<i>mcyB</i> potential/ <i>mcyD</i> potential for indicated mo			
				June	July	Early August	Late August
West, 2001							
Fort Niagara	L	3	0.011	-/+	+/+	-/-	
Wilson Tuscarora	E	3	0.014	-/-	+/+	-/-	
Town of Olcott	L	2	0.005	-/-	-/-		
Golden Hill	L	3	0.011	-/-	-/-	-/-	
Central, 2001							
Lakeside Beach	L	1	0.002			-/-	
Oak Orchard Harbor	L	2	0.013	-/-	-/-		
Hamlin	E	3	0.021	-/-	-/+	-/-	
Braddock Bay	E	2	0.010	-/-	-/-		
Eastman Park	L	2	0.010	-/-	-/-		
Irondequoit Bay	E	5	0.015	-/-	-/-	-/-	+/+
B. Forman County Park	L	2	0.005	-/-		-/-	
West Sodus Bay	E	5	0.004	-/-	-/-	-/-	-/-
Southeast, 2001							
Port Bay West	E	4	0.006	-/-	+/+	-/-	-/+
Port Bay East	L	4	0.070	-/-	-/-	-/-	+/+
Little Sodus Bay, lakeside	L	5	0.060	-/-	-/-	-/-	+/+
Little Sodus Bay, bayside	E	3	0.004			-/-	-/+
Fair Haven	E	2	0.005	-/-	-/-		
Oswego Port Authority	L	3	0.004	-/-	-/-	-/-	
Nine Mile	L	5	0.029	-/-	-/-	-/-	+/+
Little Salmon River	L	4	0.010	-/-	-/-	-/-	+/+
Selkirk Shores	L	4	0.015	-/-	-/-	-/-	+/+
Pine Grove	E	2	0.005	-/-	-/-		
East and St. Lawrence, 2001							
Sandy Pond	E	1	0.020	-/-			
Lakeview Mgmt Area	L	3	0.010	-/-	-/+	-/-	
Lakeview DEC Launch	E	1	0.003	-/-			
Westcott Beach	E	3	0.006	-/-	-/-	-/-	
Long Point Isthmus	L	4	0.004	-/-	-/+	-/-	-/-
Chaumont Bay	E	4	0.040	-/-	-/-	-/-	-/+
Tibbit Point	L	4	0.005	-/-	-/-	-/-	-/-
Cedar Point	E	4	0.005	-/-	-/-	-/-	-/-
Grass Point	E	3	0.003		-/-	-/-	-/-
Collins Point	E	1	0.003		-/-		
Southeast and East, 2003							
West Sodus Bay	E	1	0.003				+/+
Little Sodus Bay, bayside	E	1	0.003				+/+
Oswego River	E	1	0.293				+/+
Oswego Shore	L	2	0.990			+/+	+/+
Nine Mile	L	1	0.321				+/+
Little Salmon River	L	1	0.200				+/+
Selkirk Shores	L	1	1.043				+/+
Chaumont Bay	E	2	0.047				-/+

^a L, lakeside sample; E, embayment sample.^b For stations sampled more than once, only the maximum MC value is given.

quences obtained from GenBank, showed that each Lake Ontario station amplicon clustered with *Microcystis* genera and was distinctly different from *Anabaena* and *Planktothrix* sequences (Fig. 2). This large *Microcystis* clade was split into two smaller clusters, one grouping with known *Microcystis mcyA* sequences (291 bp) and the other subset clustering with a previously reported *Microcystis*-like sequence (297 bp) isolated from Lake Erie (37). This unique clade was characterized by a 6-bp insert at positions 258 to 263 in the partial *mcyA* sequence, corresponding to the same positions where additional

residues are found in *Planktothrix*, *Anabaena*, and *Nostoc* partial *mcyA* sequences. Amplicons from Chaumont Bay (eight of eight), Long Point Isthmus (four of four), Little Sodus Bay (bayside) (five of five), and Selkirk Shores (four of eight) all had clones containing this unique sequence.

Selkirk Shores, located at the mouth of a tributary running into Lake Ontario, was the only station in 2001 containing clones that clustered in both *Microcystis* clades. Samples collected from two stations at Little Sodus Bay, bayside and lakeside, had distinctively different genotypic populations. The bay-

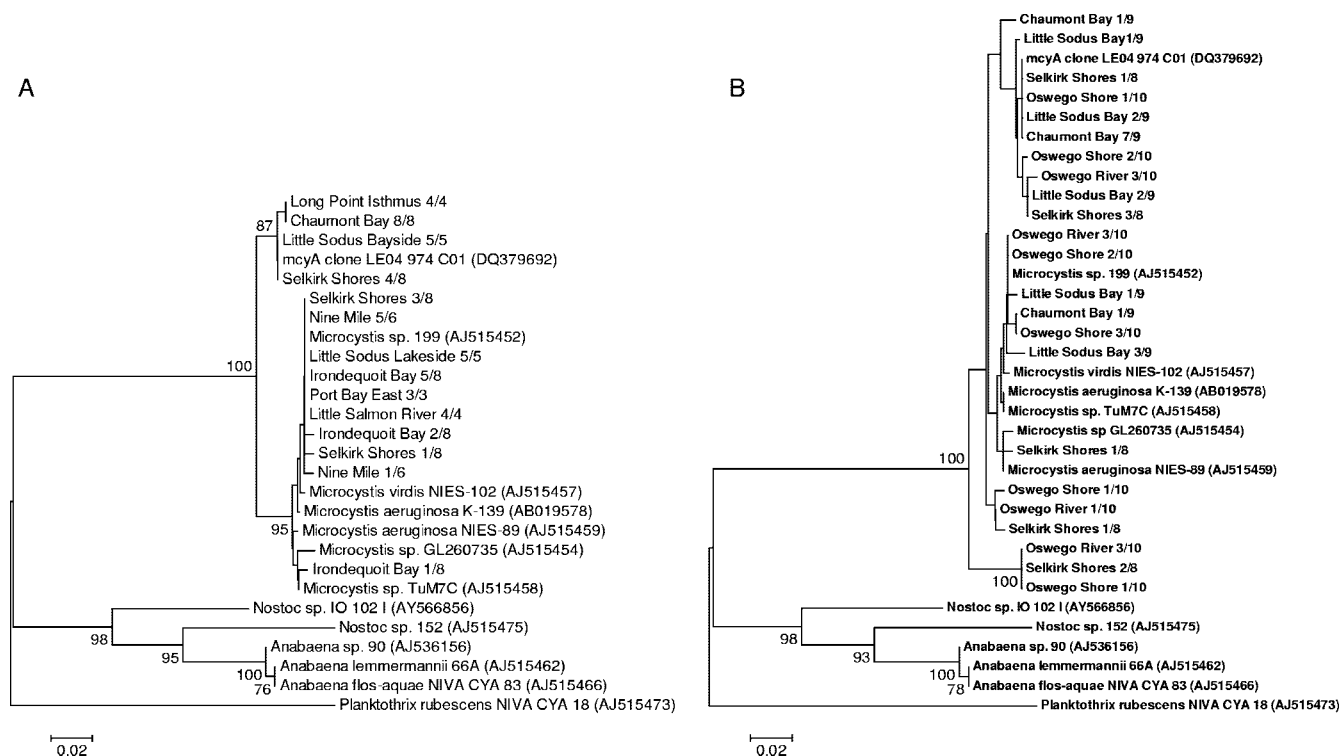


FIG. 2. Neighbor-joining trees made using *p*-distance analysis to show the relationship between partial *mcvA* genes (~300 bp) from Lake Ontario embayments in 2001 (A) and 2003 (B). Trees were generated using the program Mega 3.1. Bootstrap values of >75% are displayed at the nodes (1,000 replicates). The scale bar is a measure of the number of substitutions per site. Clone names indicate the embayment and number of clones represented. Reference sequences were obtained from GenBank, with accession numbers shown in parentheses.

side population, collected from inside the bay, corresponded entirely with the unique *Microcystis*-like *mcvA* sequence, while the lakeside population, collected from the side of the isthmus open to Lake Ontario, grouped with the traditional *Microcystis* *mcvA* sequence. Sequences obtained from the southern shore of Lake Ontario (Irondequoit, Little Sodus Bay [lakeside], Nine Mile, Port Bay East, Little Salmon River, and Selkirk Shores) formed a large cluster with other known *Microcystis* *mcvA* sequences and had comparable similarities.

In 2003, samples from Little Sodus Bay (bayside), Selkirk Shores, Chaumont Bay, Oswego Shore, and Oswego River were amplified by the *mcvA* primer set and the amplicons cloned and sequenced to determine genotype composition and the influence of lakeside and embayment populations on MC potential within Lake Ontario. A BLASTX search and phylogenetic analysis indicated that the partial *mcvA* sequences isolated were from the MC synthetase complex and again correlated with other known *mcvA* sequences from *Microcystis* genera. The large *Microcystis* clade was split into two smaller subclades, one corresponding to known *Microcystis* *mcvA* sequences and the other to the unique *Microcystis*-like *mcvA* sequences isolated in 2001 (Fig. 2). Chaumont Bay (8 of 9), Selkirk Shores (4 of 8), Little Sodus Bay (bayside) (5 of 9), Oswego River (3 of 10), and Oswego Shore (3 of 10) samples all had clones corresponding to the unique *Microcystis*-like *mcvA* sequence. Each station had a mixed genotype population.

To ensure that our identification of the genus responsible for

MC production was not due to cloning biases, species-specific *mcvE* primer sets were used. These primer sets have the same forward primer, with specific *Microcystis*, *Anabaena*, and *Planktothrix* sp. reverse primers (Table 1) (36). All stations were positive by the *Microcystis*-specific *mcvE* primer set. In 2001, four of the nine stations examined had faint amplification by either the *Anabaena* or the *Planktothrix* sp.-specific *mcvE* primer sets: Little Sodus Bay (lakeside) and Selkirk Shores showed amplification of *Planktothrix* specific genes, and Irondequoit Bay, Selkirk Shores, and Chaumont Bay amplified *Anabaena*-specific genes (data not shown).

Genotype composition and MC variants. MC variants were identified by LC-MS and compared with respect to genotype composition determined by *mcvA* sequencing (Table 3). In 2001, eight of the nine stations contained detectable levels of MC by LC-MS. The MC congeners identified included RR, YR, LR, LA, and LY (Table 3). All stations with detectable levels of MC contained the LR variant. The LR variant was dominant at all stations except Port Bay East, where the ratio of RR to LR was 51% to 49%. In 2003, four of the five samples examined had detectable levels of MC by LC-MS and included the toxin congeners RR, LR, and LA. As in 2001, all samples examined in 2003 contained the LR variant, which was also the dominant congener (Table 3). Variation in MC congener composition at both Chaumont Bay and Selkirk Shores was higher in 2003 than in 2001. Samples containing the unique *Microcystis*-like *mcvA* sequence in 2001 and 2003 had the MC variants LR, LA, and RR. In neither year did there appear to be a pattern between the observed MC

TABLE 3. MC concentration and variants, PCR results, abundance of potential MC-producing cyanobacterial cells, and trophic status at stations analyzed for genotype composition by *mcyA* sequencing^a

Yr and site	MC-LR _{eq} concn (µg/liter) ^b	MC variant ratio (%)	PCR results for CYA/MIC	% abundance of potential MC-producing cyanobacterial cells ^c of indicated genus			Carlson's trophic status index for:		Trophic status
				<i>Microcystis</i>	<i>Anabaena</i>	<i>Aphanizomenon</i>	Chl-a	Total P	
2001									
Irondequoit Bay	0.015	LR (50)/RR (25)/LY (25)	+/+	85.9	0.0	14.1	40	34	Oligo
Port Bay East	0.007	RR (51)/LR (49)	+/+	0.0	30.9	69.1	40	58	Meso
Little Sodus Bay, bayside	0.004	LR (80)/LA (20)	+/+	86.0	0.0	14.0	48	18	Oligo
Little Sodus Bay, lakeside	0.060	LR (81)/YR (13)/RR (6)	+/+	93.7	0.5	5.8	38	43	Meso
Salmon River	0.004	LR (100)	+/+	0.0	0.0	0.0	42	63	Eu
Nine Mile	0.013	LR (100)	+/+	0.0	0.0	0.0	46	35	Meso
Selkirk Shores	0.015	LR (100)	+/+	0.0	0.0	0.0	41	24	Oligo
Long Point Isthmus	0.004	ND	+/+	100.0	0.0	0.0	59	64	Eu
Chaumont Bay	0.040	LR (100)	+/+	0.0	0.0	0.0	33	28	Oligo
2003									
Little Sodus Bay, bayside	0.003	ND	+/-	18.5	71.2	10.3	36	58	Meso
Chaumont Bay	0.047	LR (72)/LA (28)	+/+	5.1	94.9	0.0	46	47	Meso
Selkirk Shores	1.043	LR (78)/RR (22)	+/+	100.0	0.0	0.0	50	76	Eu
Oswego Shore	0.769	LR (72)/RR (28)	+/+	100.0	0.0	0.0	48	68	Eu
Oswego River	0.239	LR (100)	+/+	100.0	0.0	0.0	42	61	Meso

^a Samples listed in bold have the novel *mcyA* insert. ND, not detected; Chl-a, chlorophyll *a*; eu, eutrophic; meso, mesotrophic; oligo, oligotrophic.^b MC concentration from the sample cloned.^c No *Planktothrix* cells were observed.

variant and the presence or absence of the unique *Microcystis*-like *mcyA* sequence (Table 3).

Trophic status and cyanobacterial species composition. The Carlson trophic status index and cyanobacterial species composition were compared to those for samples targeted by *mcyA* sequencing in 2001 and 2003. Trophic status, based on average chlorophyll *a* and total phosphorous trophic status indices, ranged from oligotrophic to eutrophic in 2001 and mesotrophic to eutrophic in 2003 (Table 3). In 2001, there was no relationship between MC concentration and trophic status ($r^2 = 0.16$, $n = 9$), while in 2003, the samples examined exhibited higher MC production with increased trophic state ($r^2 = 0.99$, $n = 5$). Stations containing the unique *Microcystis*-like *mcyA* sequence in 2001 and 2003 ranged from oligotrophic to eutrophic.

Cyanobacterial cell numbers fluctuated widely between sampling stations in both 2001 and 2003. Several stations had few or no visible *Microcystis* cells (five of nine stations in 2001), but amplicons were obtained by the cyanobacterial and *Microcystis* sp.-specific primer sets. In 2003, Selkirk Shores and Oswego Shore, two stations corresponding to particulate MC concentrations near the WHO guideline value for MC in drinking water, had few potentially toxic cyanobacteria present by microscopy in the net-collected samples. There were *Anabaena* filaments present in 4 of the 13 stations counted, but no *Planktothrix* filaments were observed. In general, the abundance of potentially toxic cyanobacterial genera was low. Stations containing the unique *mcyA* genotype contained either no visible toxigenic cyanobacterial cells (Selkirk Shores and Chaumont Bay in 2001), only *Microcystis* cells (Long Point Isthmus in 2001 and Selkirk Shores, Oswego River, and Oswego Shore in 2003), a combination of *Microcystis* and *Anabaena* cells (Chaumont Bay in 2003), a combination of *Microcystis* and *Aphanizomenon* cells (Little Sodus Bay [bayside] in 2001), or a combination of *Microcystis*, *Anabaena*, and *Aphanizomenon* cells (Little Sodus Bay [bayside] in 2003).

DISCUSSION

Embayments such as Irondequoit, Port, Little Sodus, Chaumont, and the Oswego River are significantly separated from each other and the main body of Lake Ontario and are considered separate systems. They are affected by cultural eutrophication and provide protected mesocosms for cyanobacterial growth and potential MC production (23). The eutrophication is in part due to sewage effluent (Port Bay), high turbidity (Little Sodus Bay), river discharge (Oswego Harbor), or agricultural runoff and inadequate septic systems (Chaumont Bay). Makarewicz et al. (24) explored MC production along the southern shore of Lake Ontario from the Niagara River to Chaumont Bay and reported levels well below the WHO guideline value for drinking water of 1.0 µg MC-LR_{eq} liter⁻¹ (maximum MC concentration of 0.795 µg liter⁻¹ at Long Pond North), with the highest MC values from bays and rivers (24). More recently, Makarewicz et al. (unpublished) have reported an algal scum (Sackets Harbor) and shore-side sample (Oak Orchard Creek) containing higher MC concentrations. Here, we report MC levels from Oswego Shore (near the site of the Onondaga County water intake pipes) and Selkirk Shores (a public park area) with MC values near 1.0 µg MC-LR_{eq} liter⁻¹. MC values reported for the 32 other stations (Fig. 1) were well below 1.0 µg MC-LR_{eq} liter⁻¹ (Table 2); however, the potential for MC production was widespread (see below).

Cyanobacteria and *Microcystis* spp. were present in nearly all of the samples collected in 2001 and 2003 as determined by molecular analysis. Microscopic examination revealed few cyanobacteria, including *Microcystis* spp., at several sites. This may be a reflection of low cell abundance and phycological sampling technique in areas corresponding to high sediment loading and water mixing. Cyanobacterial cells and small colonies can have diameters of less than 63 µm, thereby passing through plankton nets, leading to underestimates of the community population. The lack of visual confirmation emphasizes the

need for molecular analysis to detect potentially toxic cyanobacteria in low concentrations prior to formation of blooms with elevated toxin levels. PCR amplicons from the *mcyB* and *mcyD* primer sets indicated the potential for toxin formation along the southern and eastern shores of Lake Ontario (17 of 34 stations). The lack of toxin genes in early August 2001 indicated periodicity in toxin blooms similar to previously reported bloom dynamics in New York State (15). The *mcyD* primer set detected MC genes more often than the *mcyB* primer set and was more applicable to detecting toxin genes at low concentrations. Sensitivity studies comparing the *mcyD* and *mcyB* primer sets confirm this finding and support the use of primers against *mcyD* as an early indicator of toxin potential (A. M. Hotto, unpublished data).

Microcystis was the major MC producer in Lake Ontario based on *mcyA* sequences. It is possible that the *mcyA* primers reflected only the dominant MC sequences from each station, thereby underestimating the genotypic diversity within the population. However, use of *mcyE* species-specific primers confirmed the presence of *Microcystis* spp. and indicated that the presence of potentially toxic *Planktothrix* and *Anabaena* species was not widespread. *Anabaena* and *Oscillatoria* spp. have been previously detected in offshore waters of Lake Ontario; however, they did not make up a large proportion of the phytoplankton biomass (<15%) (22). This was in agreement with our current observations.

The appearance of a unique *Microcystis*-like *mcyA* sequence in Lake Ontario is still perplexing. The sequence was characterized by a 6-bp insert (TTTGCG), corresponding to the amino acids Phe-Ala (FA). This is similar to the insertion in the partial *mcyA* sequences of *Anabaena* and *Nostoc* spp. (TTTGGT; FG) and *Planktothrix* spp. (TTGGGT; LG) and observed in samples from Lake Erie (TTTGCG; FA) (37). It is unknown whether this genotype represents a novel *Microcystis* sp. or a genus closely related to *Microcystis* that has not been previously reported to produce MCs. Efforts to isolate the responsible species are currently in progress. The presence of this genetic element may be due to a recombination event, a mutational insertion, or an ancestral relic. It is most closely related to a similar insertion in the partial *mcyA* sequences of *Anabaena* and *Nostoc* spp., lending support to reports that the MC biosynthetic gene cluster originated in an *Anabaena*-like sp. due to the colinearity between the *mcy* genes and MC structure (4, 25, 38). It is unknown how the extra base pairs and their corresponding amino acid residues affect MC production or toxicity.

Variations in trophic status and cyanobacterial species composition were examined to determine if they corresponded to the presence or absence of the unique *mcyA* genotype. The unique *mcyA* genotype was present in samples ranging from oligotrophic to eutrophic, in stations containing few or no visible potentially toxic cyanobacterial cells, and in stations containing *Microcystis*, *Anabaena*, and *Aphanizomenon* species. There was increased genotypic diversity in Chaumont Bay and Little Sodus Bay (bayside) from 2001 to 2003 concurrent with the visible appearance of *Anabaena* filaments. However, there was no indication of toxic *Anabaena* in 2003 by either *mcyA* sequencing or *mcyE* species-specific amplification. The lack of congruity between nutrients, biomass, cyanobacterial compo-

sition, and toxicity emphasizes the unpredictable nature of algal blooms.

Variation within the MC congener profile was examined to determine its relationship with the presence/absence of the unique *mcyA* sequence. Previously, only the MC-RR, -YR, and -LR variants have been detected in Lake Ontario (29). Here, we also identified MC-LA and -LY, but the most common congener was MC-LR, corresponding to both *mcyA* genotypes. Variations were not observed in the Ala residue encoded by the *mcyA2* gene targeted by our *mcyA* primer set. Increased genetic diversity from 2001 to 2003 coincided with the appearance of MC congeners MC-LA (Chaumont Bay) and -RR (Selkirk Shores).

One of the key questions for the management of MC-producing species is whether a toxic bloom originates in an embayment and is transported to the lake or initiates offshore and is transported through the lake via the lakewide circulation pattern. The sporadic distribution of *mcy* potential along the shoreline (Table 2) indicates that separate stations harbor potential MC-producing populations. The separation of genotype and phenotype between most embayments and lakeside samples confirms that the water current pattern did not have a strong effect on MC distribution. This is also supported by the spatial differences in MC concentration in both this study and reports by Makarewicz et al. (unpublished). Water exchange between the embayment and the main lake may result in the export of organisms to the lake and their transport along the shoreline by prevailing water currents. However, embayments such as Little Sodus Bay are separated from Lake Ontario such that water exchange is negligible (40).

Lakeside genetic populations examined along the southern shore of Lake Ontario in 2001 (Little Sodus Bay [lakeside], Nine Mile, Port Bay East, Little Salmon River, and Selkirk Shores) clustered together, representing low genotypic diversity. The absence of the unique *mcyA* genotype from the lakeside samples indicated that this genotype originated in selected embayments. Selkirk Shores was the only station with a mixed genotypic population in 2001. The traditional *mcyA* genotype could have originated upstream in the embayment or been carried in from the west, while the unique *mcyA* genotype likely originated in the protected embayment. The disparity between genotype populations at Little Sodus Bay in 2001 and 2003 may be a result of water runoff carrying another toxin population into the embayment. Storm-induced currents have the ability to flush toxic genera into the lake from nearby ponds and small lakes due to the strong affect on particle flow (up to several tens of cm s^{-1}), resulting in unusual mixing patterns and the introduction of different MC genotypes (3). Makarewicz et al. (unpublished) also noted higher MC concentrations in ponds, rivers, lakes, and embayments draining into Lake Ontario, suggesting that these sources serve as a reservoir for MC production.

In 2003, the Oswego River strongly influenced the MC-producing population. The Oswego River and Oswego Shore samples had similar genotype compositions, indicating that the toxin population originated upstream or at the mouth of the Oswego River and was carried into the lake. The same traditional and novel *mcyA* sequences have been detected in Oneida Lake, which could seed Lake Ontario through the Erie Canal and Oswego River (A. M. Hotto, unpublished). We

hypothesize that the cyanobacterial population was then transported eastward to the Oswego Shore site. This circulation pattern may also have transported the population to Selkirk Shores and contributed to the high MC content observed there. It is possible that transportation of this unique genotype between lakes may occur through external factors, such as fishing boat transfers or animal carriers. The observed novel sequence has been noted only in the northeastern United States, giving support for the formation of a unique ecotype.

Although MC concentrations at most stations were below the WHO guideline value for drinking water, MC-producing genotypes were widespread along the New York shoreline of Lake Ontario. Distribution of a unique MC-producing genotype indicated that Lake Ontario embayments harbor toxigenic cyanobacteria. Toxin populations from lakeside stations add to the cyanobacterial populations along the shoreline, and river inputs have a large influence on mixed genotypic populations. MC production and genotype distribution were not correlated with nutrients or algal biomass. Continued alteration of shoreline habitat, introduction of nonnative species, and decline of native community populations in Lake Ontario, where the genes for toxin production have been shown to exist, may all lead to increased occurrence of cyanobacterial blooms and MC production in embayments with elevated nutrient levels, increased light penetration due to zebra mussel introduction, and protection from wave turbulence. It is essential that monitoring programs be aware of the presence of these potential MC producers and the novel genotypes found in these lakes.

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